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Abundance and microhabitat use of the Endangered toad *Rhinella yanachaga* (Anura: Bufonidae) in the cloud forest of Yanachaga Chemillén National Park, Peru

[1,2]Shirley Huamán-Trucios, [3]Vladimir Camel, [1]Edith Orellana Mendoza, [4]Marcela V. Pyles, and [5,*]Rudolf von May

[1]*Facultad de Ciencias Forestales y del Ambiente, Universidad Nacional del Centro del Perú, Huancayo, PERÚ* [2]*Servicio Nacional de Áreas Naturales Protegidas por el Estado - SERNANP, Lima, PERÚ* [3]*Universidad Científica del Sur, Lima, PERÚ* [4]*Biology Institute, Sector of Ecology and Conservation, Federal University of Lavras (UFLA), Minais Gerais, BRAZIL* [5]*Biology Program, California State University Channel Islands, Camarillo, California, USA*

***Abstract.*—The arboreal toad *Rhinella yanachaga* is an endemic species of the cloud forest of central Peru, and is categorized as Endangered according to the International Union for Conservation of Nature. The core habitat of this species is within the Yanachaga-Chemillén National Park, but the status of its populations remains unknown. Obtaining quantitative data based on field surveys is essential for conserving this species in the park. In this study, the abundance, size, and microhabitat use of *R. yanachaga* were examined across an elevational gradient. Individuals with snout-to-vent length (SVL) ≥ 20 mm were sampled in four transects between 2,400 and 2,800 m, in the wet and dry seasons. Using night surveys, individual data were recorded on sex, SVL, microhabitat, geographic location, relative humidity, and temperature. The abundance of females and males varied among transects in dry and wet sampling periods. We recorded more individuals in the dry season and observed that frogs distributed at higher elevations tend to have a larger body size than those at lower elevations. Most individuals appear to prefer microhabitats composed of leaves and ferns. Additionally, we observed sexual dimorphism in size, as females were larger than males. These findings contribute to amphibian conservation programs in Peru.**

Keywords. Amphibian, population status, elevational gradient, endemic species, habitat, South America.

***Resumen.*—El sapo arbóreo *Rhinella yanachaga* es una especie endémica del bosque nuboso del centro del Perú y está clasificada como En Peligro según la Unión Internacional para la Conservación de la Naturaleza. El hábitat principal de esta especie se encuentra dentro del Parque Nacional Yanachaga-Chemillén, pero el estado de sus poblaciones es desconocido. La recopilación de datos cuantitativos de estas poblaciones es esencial para conservar la especie en el parque. En este estudio, se examinó la abundancia, tamaño y el uso de microhábitat de *R. yanachaga* en un gradiente de elevación. Se muestreó individuos con longitud de hocico-cloaca (LHC) ≥ 20 mm en cuatro transectos distribuidos entre 2,400 y 2,800 msnm, en la estación húmeda y seca. Mediante monitoreos nocturnos, registramos datos individuales sobre sexo, LHC, microhábitat, ubicación geográfica, humedad relativa y temperatura. Observamos que la abundancia de hembras y machos varió entre los cuatro transectos en ambos períodos de muestreo. Registramos más individuos a mayor altitud y en la estación seca, además observamos que los individuos distribuidos a mayor elevación tienden a ser más grandes que individuos distribuidos a de elevaciones más bajas. La mayoría de los individuos parecen preferir el microhábitat compuesto de hojas y helechos. Además, observamos dimorfismo sexual en tamaño, en donde las hembras fueron más grandes que los machos. Estos hallazgos contribuyen a los programas de conservación de anfibios en Perú.**

Palabras clave. Anfibio, estado poblacional, bosque de neblina, gradiente de elevación, especie endémica, hábitat, América del Sur.

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Correspondence. **rvonmay@gmail.com* (RVM), *melanny.ht@gmail.com* (SHT), *vlad_camel@hotmail.com* (VC), *eporellana@uncp.edu.pe* (EOM), *marcela.pyles@gmail.com* (MVP)

Introduction

The cloud forests of Yanachaga-Chemillén National Park (YCNP), located in the eastern region of the central Andes of Peru, are considered a hotspot of biodiversity (Young 2007; Myers et al. 2000). The park is the core of the larger Oxapampa-Ashaninka-Yanesha Biosphere Reserve, which aims to maintain cultural and biological diversity, ecosystem function, and sustainable use of natural resources in the region (Griesinger 2019). The YCNP and the buffer zone surrounding the park protect the habitat of over 40 species of amphibians (Chávez et al. 2012; Angulo et al. 2016). Most of these species are known from a small geographic area and are vulnerable to habitat loss and disease (Aguilar et al. 2010; Chávez et al. 2012; Jarvis et al. 2015). Conservation of key areas within the YCNP and its buffer zone is a priority, because they contain the only known populations of these endemic amphibians (Lehr and von May 2004; Boano et al. 2008; Angulo et al. 2019). Obtaining quantitative data based on field surveys is essential for the monitoring and protection of the species living in the YCNP.

One of these endemic species is *Rhinella yanachaga* Lehr, Pramuk, Hedges, and Córdova, 2007, an arboreal toad (Bufonidae) distributed from 1,814 to 2,900 m asl in the cloud forest of YCNP (Lehr et al. 2012). *Rhinella yanachaga* is a medium-sized toad with nocturnal habits, reaching a maximum snout-to-vent length (SVL) of 45.7 mm (Lehr et al. 2007). The species is currently categorized as Endangered according to the International Union for Conservation of Nature (IUCN) *Red List of Threatened Species* (IUCN 2018), and is currently known from only two localities—one inside the YCNP and one in the buffer zone (outside) of the YCNP (Chávez et al. 2012). Surveys of threatened amphibians such as *R. yanachaga* are a priority for the park's biodiversity monitoring program, and population status assessments conducted every 10 years are a priority for global assessments (The Rules of Procedure for IUCN Red List Assessments 2017–2020; IUCN 2016). Therefore, it is necessary to have data on the abundance and microhabitat use of this species.

Here, we present data on the abundance and microhabitat use of *R. yanachaga* across an elevational gradient in a cloud forest in the YCNP. Additionally, given that seasonality may affect the activity and abundance of a species, we compared the relative abundance and size of *R. yanachaga* between the wet and dry seasons along the elevational gradient. Whether the number of individuals and size of *R. yanachaga* vary across elevation, and whether seasonality in temperature affects the habitat use of this species were also assessed. This work provides data that can be used to further understand the effects of environmental variables on the distribution of threatened amphibian populations in the Tropical Andes (Larsen et al. 2012).

Materials and Methods

Study Area

This study was carried out in the cloud forest sector of San Alberto within the Yanachaga-Chemillén National Park, Oxapampa Province, Pasco Department, Peru (Fig. 1). The study area is within the tropical montane forest ecoregion, locally known as *Selva Alta* as defined by Brack (1986). Additionally, the area includes three life zones as defined by the Holdridge system (ONERN 1976

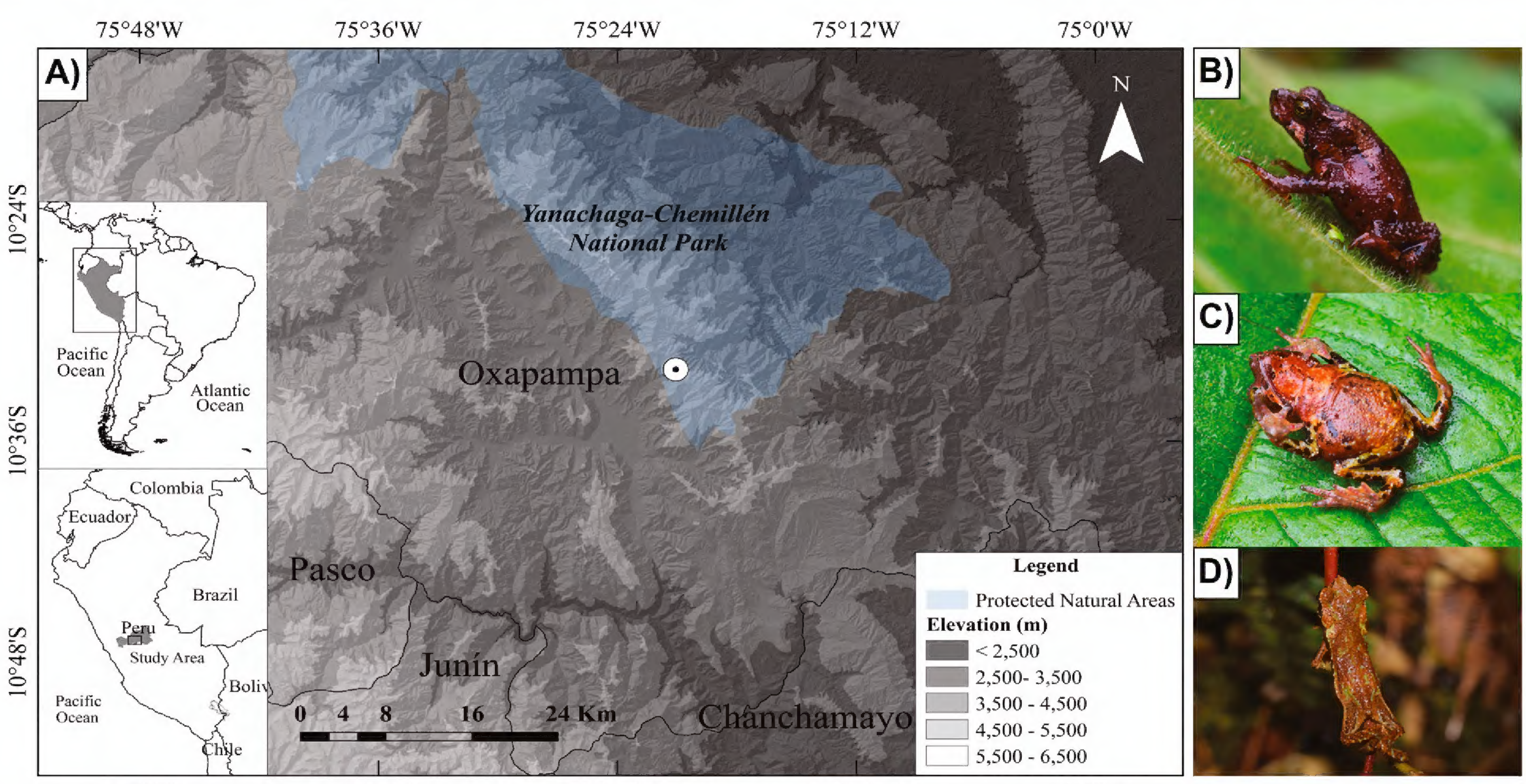

Fig. 1. (A) Map of the study area. The white circle indicates the location of the study site (San Alberto) within Yanachaga-Chemillén National Park, Oxapampa, Pasco; **(B)** lateral view, **(C)** ventral view, and **(D)** dorsal view of *Rhinella yanachaga* in life. Map by Vladimir Camel **(A)**, photos by Shirley Huamán-Trucios **(B–D)**.

[ONERN = *Oficina Nacional de Evaluación de Recursos Naturales*]): Very Humid Low Montane Tropical Forest, Low Montane Tropical Rain Forest, and Low Montane Tropical Humid Forest.

In the study area, in the dry season the level of humidity is 89.1% ± 5.16 and the average temperature is 11.7 °C ±1.55, while the wet season has lower humidity levels (80.3% ± 4.57) and a higher average temperature (14.2 °C ± 1.87).

Data Collection

Four linear transects of 1,550 m × 2 m (length × width) were established. Each transect was located at one of the following elevational bands (in m): 2,400–2,500, 2,500–2,600, 2,600–2,700, and 2,700–2,800. Data were collected using visual encounter surveys at night (1900 to 0000 h) during both the wet season (January–March) and the dry season (June–July) in 2018. Each transect was surveyed three times per season. Standard biosafety protocols were followed to avoid the introduction and spread of pathogens (Angulo et al. 2006). Protocols included the disinfection of gloves with 70% ethyl alcohol after measuring the morphological characteristics of each individual. Additionally, the field equipment, including boots, was disinfected in a solution of 4% sodium hypochlorite (4 mL) diluted in 1 L of water. Likewise, field instruments, such as the GPS, vernier, and flashlights, were disinfected with 70% ethyl alcohol.

Three observers participated in each night survey, and captured the individuals of *R. yanachaga* observed in each transect. For each individual of *R. yanachaga* ≥ 20 mm SVL, the sex, SVL, and elevation were recorded following the methodology proposed by Lips and Reaser (1999). The sex of each individual was determined by examining external characters (Lehr et al. 2007): males have hypertrophied forearms and females have slim forearms; the cloaca in males is more protuberant and ventrally oriented than in females. The following data were recorded at each capture point: transect location, coordinates, and microhabitat type (leaves, bromeliads, ferns, orchids, or moss).

With regards to forest microhabitat categories, the types considered were arboreal, shrub, and herbaceous vegetation. The main groups of epiphytes were recorded, including orchids and bromeliads, as well as ferns and moss. The orchids evaluated included *Epidendrum* sp., and epiphytic bromeliads included *Guzmania jaramilloi*, *Guzmania melinonis*, *Aechmea zebrina*, and *Tillandsia* sp. Ferns included *Elaphoglossum* sp. and *Campyloneurum* sp., and the moss microhabitat included *Sphagnum magellanicum*.

Data Analysis

First, the frequency distribution of individuals (males and females) was visually assessed according to four altitudinal classes (2,400–2,500, 2,500–2,600, 2,600–2,700, and 2,700–2,800 m). Subsequently, Generalized Additive Models (GAM) were used to examine the relationship between abundance and elevation. The numbers of male and female individuals found in the two seasons (dry and wet) were grouped. Subsequently, Generalized Linear Mixed Models (GLMM) were used to examine how size varies as a function of four explanatory variables (microhabitat, elevation, sex, and season) and three interaction effects (elevation-microhabitat, microhabitat-sex, and sex-season). Given the possible lack of independence between the individuals sampled, the transects and the sampling time were considered as random factors. The variance homogeneity was verified by means of residual graphs. Differences in size with respect to seasons, sex, and microhabitat were accessed by Tukey's post hoc tests. A Gamma distribution function with log link was used to fit the model of size. The effects and significance of each variable on *R. yanachaga* size were accessed from a multi-model inference approach based on Akaike's Information Criterion with a correction for small sample size (AICc) (Burnham and Anderson 2002). Competing models with delta AICc < 2 were used in conditional model averaging, and averaged parameter estimates were presented as the final result of the modeling.

Results

During the study, 226 individuals of *R. yanachaga* were recorded, including 103 individuals found in the wet season and 123 individuals in the dry season. The highest abundance in the wet season was recorded in the elevation range of 2,700 to 2,800 m, while the highest abundance in the dry season was recorded at elevations of 2,600 to 2,700 m (Fig. 2). In both seasons, the lowest abundance of *R. yanachaga* was recorded at lower elevations (Fig. 2C). Overall, more males (171) than females (54) were found during this study (Fig. 2). Additionally, larger individuals (>32 mm) were less frequent than small and mid-sized individuals. Most male individuals had recorded SVL ranging between 24 and 28 mm, whereas females presented a higher frequency within the range of 28 to 32 mm (Fig. 3).

Individuals of *R. yanachaga* were found to have a higher preference for ferns and leaves of shrubs compared to bromeliads (Table 1). Both females and males were found to use similar microhabitats, and there were no significant differences in the abundance between dry and wet periods (Appendix 2). The abundance of individuals of both sexes did vary as a function of elevation. Likewise, the regression analysis showed that elevation has a positive effect on the size of *R. yanachaga* individuals (Table 2), particularly when considering sex as a determining factor (Table 2). The females tended to be larger at higher elevations ($r^2 = 0.34$, $p = 0.01$), but male body size was not correlated with elevation ($r^2 =$

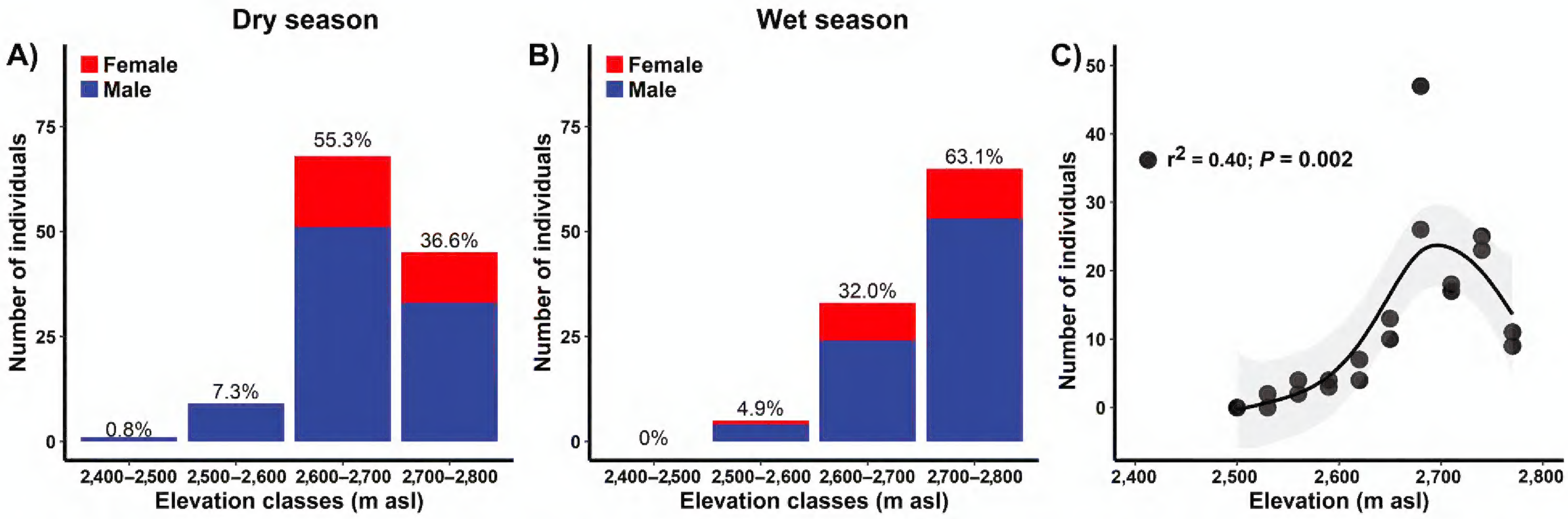

Fig. 2. Number of *Rhinella yanachaga* individuals per transect, T1 = 2,800–2,700 m, T2 = 2,700–2,600 m, T3 = 2,600–2,500 m, and T4 = 2,500–2,400 m, according to the elevation gradient for both sexes, in **(A)** dry season and **(B)** wet season. **(C)** Correlation between the abundance of *Rhinella yanachaga* and elevation for both sexes and seasons. The grey band indicates the 95% confidence limits.

0.003, $p = 0.41$) (Fig. 4).

Moderate variation in body size of *R. yanachaga* was observed between seasons (Fig. 5A–B). Overall, females (= 31.31 mm ± 0.82) tend to be larger than males (= 26.22 mm ± 0.50, Fig. 5C). Lastly, average body size of *R. yanachaga* varied among microhabitats; individuals found on bromeliads were larger than those on leaves or ferns (Fig. 5D).

Discussion

The results support our prediction that relative abundance and microhabitat use of *Rhinella yanachaga* vary across an elevational gradient in the cloud forest of central Peru. Given that this ecosystem is characterized by high humidity, provided by fog and rainfall throughout the year, it supports unique habitats and microhabitats used by endemic amphibian species (Duellman and Lehr 2009). However, to date, the ecological drivers affecting the distribution of the genus *Rhinella* have remained unclear.

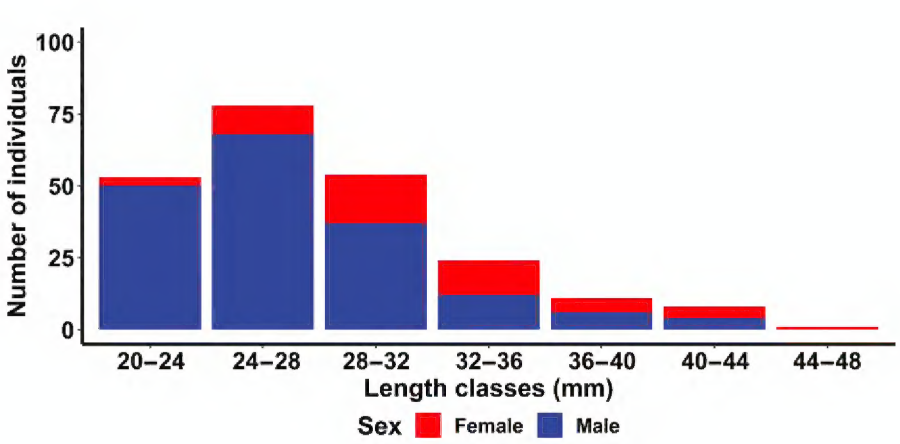

Fig. 3. Numbers of individuals according to sex and length (SVL) ranges of *Rhinella yanachaga* in four transects, T1 = 2,800–2,700 m, T2 = 2,700–2,600 m, T3 = 2,600–2,500 m, and T4 = 2,500–2,400 m, according to the elevation gradient in the wet and dry seasons.

The results suggest that relative abundance of *R. yanachaga* does not vary across seasons. Several studies have shown that anurans are more abundant during the wet season, as higher precipitation favors their development and reproduction (Arroyo et al. 2003; Ceron et al. 2020; Linause et al. 2020; Ortega et al. 2011; Narvaes et al. 2009; Zaracho and Lavilla 2015). For example, in Venezuela, during the high precipitation season, *Hyalinobatrachium duranti* showed increased abundance in a cloud forest (Villa et al. 2019). However, we found no significant differences between seasons. This probably reflects the constant presence of mist and cloudiness, which results in reduced solar radiation, mesic temperatures, and increased relative humidity variables that favor amphibian activity throughout the year (Segev et al. 2012). Similar patterns have been observed in terrestrial breeding frogs in the genus *Pristimantis* (*P. miyatai*, *P. douglasi,* and *P. merostictus*) in another Andean cloud forest (Arroyo et al. 2003).

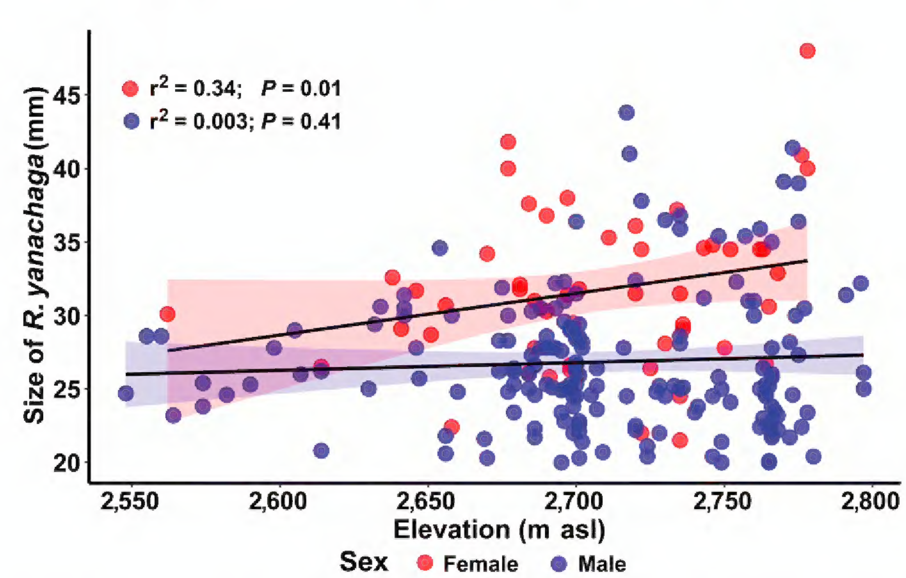

Fig. 4. Correlations between the elevation and size of *Rhinella yanachaga* according to sex. The model presents correlation factors of $r^2 = 0.34$; $p = 0.01$ for females and $r^2 = 0.003$; $p = 0.41$ for males. The colors indicate sex (blue for males and red for females) and the shaded areas indicate the 95% confidence limits.

Table 1. Microhabitat use by *Rhinella yanachaga* in the cloud forest of Yanachaga-Chemillén National Park, Peru.

Microhabitat	Number of individuals	Number of females	Number of males
Leaf (shrub)	110	24	86
Fern	96	22	74
Bromeliad	16	5	11

The different proportions of female and male individuals across the elevational gradient, particularly those observed between 2,600 and 2,800 m at both times of the year, could be related to the availability of reproductive sites. However, further work (e.g., mark-recapture study) is needed to determine whether *R. yanachaga* exhibits site fidelity (to reproductive or retreat sites) at these elevations.

Our results agree with the study of Lehr et al. (2007), and provide evidence that *R. yanachaga* exhibits sexual dimorphism in body size, with females being larger than males. Sexual dimorphism in body size has phenotypic importance in the reproductive periods, particularly because bigger females tend to produce larger eggs or increased numbers of eggs, and because they may lay eggs more than once during a reproductive season (Rodrigues da Silva and Feres 2010). Our data also suggest that the frequency and size of *R. yanachaga* individuals increases at higher elevations. A similar trend has been observed in terrestrial-breeding frogs in the tropical Andes (von May et al. 2018; Santa-Cruz et al. 2019). This pattern supports the prediction of Bergmann's rule, in which organisms tend to be larger in colder environments than in warmer environments (Mayr 1956). The elevation gradient is one of the most important factors for life that is globally associated with air temperature, and low temperatures have an effect on species traits (Korner 2007).

Some plants, such as bromeliads, were found to support larger individuals of *R. yanachaga*, on average, than other plant structures (e.g., leaves or ferns). Climbing vegetation up to 1.5 m above ground is common in many anurans (Duellman 1978; Toft and Duellman 1979; Chávez et al. 2012). Individuals using different plant structures often remain inactive during the day and exhibit reproductive activity at night, and this behavior is similar to that of *R. yanachaga* with the exception that male individuals were found on herbaceous or shrubby vegetation. Additionally, individuals found on bromeliads were larger than individuals found on other plants, in both seasons (Table 3, Fig. 5D). Bromeliads provide a suitable microhabitat for the survival and breeding of amphibians with predominantly terrestrial habits (García et al. 2005; Jiménez-Robles et al. 2017).

Table 2. Statistical data on the relationship between elevation and size of *Rhinella yanachaga* according to GLMM. Asterisks indicate the level of significance: "*" $p \leq 0.05$; "**" $p \leq 0.01$; and "***" $p \leq 0.001$.

Interaction	Estimate	Std. error	z value	Pr (> |z|)	
(Intercept)	2.652	0.846	3.133	0.001	**
Sex	0.232	0.745	0.312	0.754	
Elevation	0.001	0.0002	2.016	0.043	*
Elevation × Sex	-0.001	0.000009	66.296	< 0.001	***

In summary, to our knowledge, this is the first study reporting the population status and microhabitat use of *R. yanachaga* at different elevations in the YCNP. We found that the relative abundance and body size of this species vary across both elevations and seasons. We confirm that *R. yanachaga* presents sexual dimorphism in size, with females larger than males. Our findings show that the abundance and size of *R. yanachaga* tend to increase at higher elevations. The increased frequency of both females and males at 2,600–2,800 m could be attributed to the terrestrial reproduction mode of *R. yanachaga*. The abundance of amphibians changes along the elevation gradient as influenced by the thermal tolerance of ectothermic species in narrow altitudinal ranges (Bernal and Lynch 2008; Jiménez-Robles et al. 2017). In addition, we found that *R. yanachaga* more frequently uses microhabitats such as leaves and ferns, but the largest individuals settle in bromeliads. Our data also indicate that 2,400–2,500 m represents the lower elevational range limit of *R. yanachaga*. Finally, given that *R. yanachaga* exhibits limited geographic and elevational distributions, this species could be used in

Table 3. Comparisons of the sizes of individuals of *Rhinella yanachaga*. Asterisks represent significant differences in the parameters with the Tukey test after GLMM: "*" $p \leq 0.05$; "**" $p \leq 0.01$; and "***" $p \leq 0.001$.

	Variable	Estimate	Std. error	t value	Pr (> |z|)	
Females	Wet season	31.9	2.3	47.86	< 0.001	***
	Dry season	28.8	2.06	-3.405	0.001	***
Males	Wet season	26	0.585	144.75	< 0.001	***
	Dry season	27.2	0.613	1.723	0.084	
Sex	Female	31.309	0.824	130.798	< 0.001	***
	Male	26.225	0.502	-6.931	< 0.001	***
Microhabitat	Bromeliad	31.319	1.442	74.784	< 0.001	***
	Fern	27.145	0.618	-3.013	0.003	**
	Leaf	27.187	0.615	-3.071	0.002	**

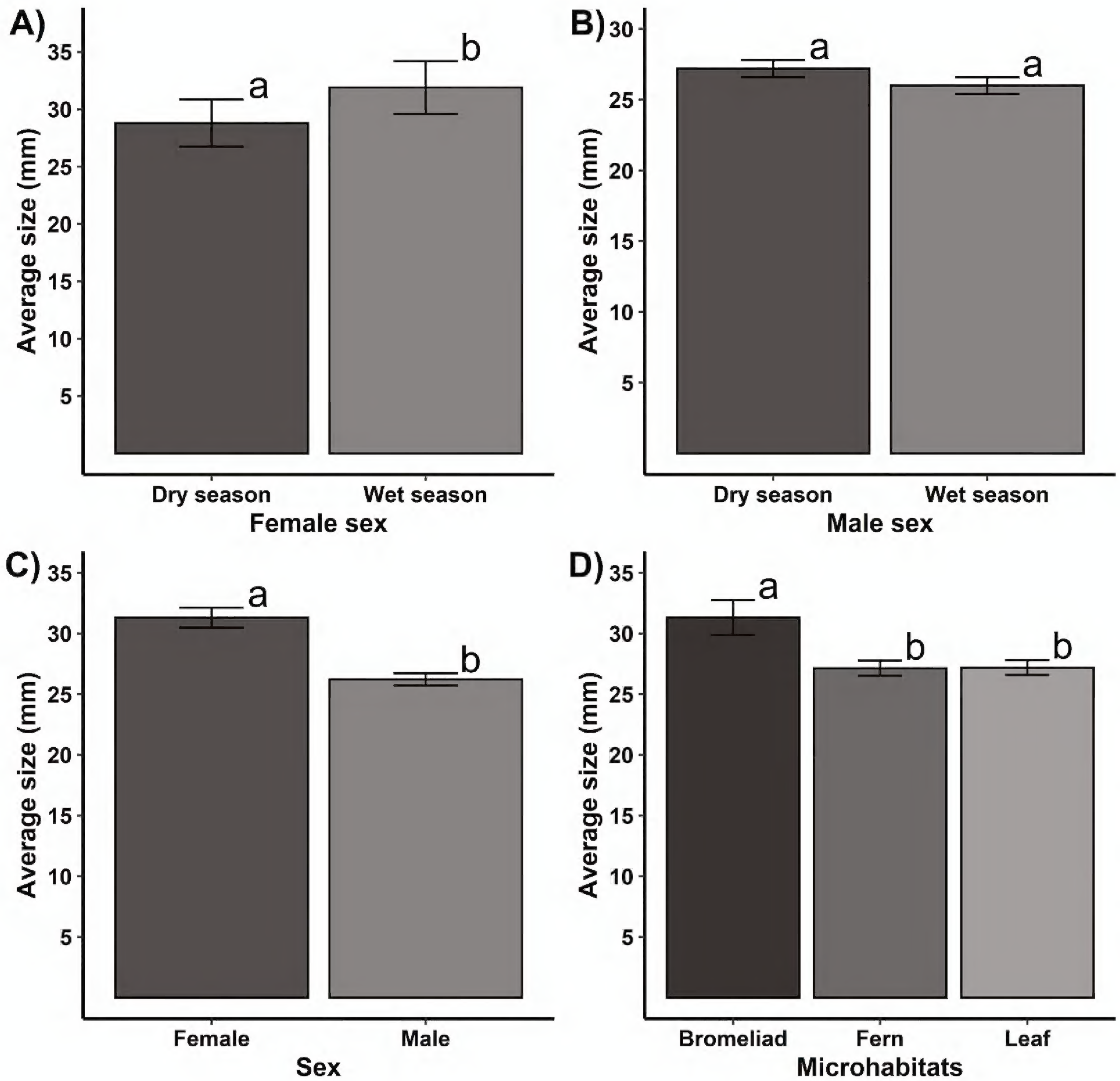

Fig. 5. Comparisons of the sizes of *Rhinella yanachaga* individuals. **(A)** Differences in size of females in dry and wet seasons. **(B)** Differences in size of males in dry and wet seasons. **(C)** Differences in size between males and females. **(D)** Differences in the size of individuals with respect to microhabitats. The different letters indicate significant differences at $p \leq 0.05$ according to the Tukey test after GLMM. Error bars represent standard errors.

further studies on local adaptation in relation to thermal physiological limits.

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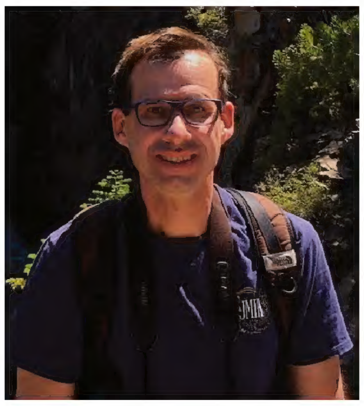
Shirley Melanny Ross Huamán-Trucios is a Forestry Engineer at the Universidad Nacional del Centro del Perú and master's student of Forest Resources Conservation at the Universidad Nacional Agraria La Molina, Lima, Perú. Currently, Shirley is an official park ranger for the National Service of Natural Protected Areas, Perú. Her main research interests are biodiversity conservation and amphibian behavioral ecology in tropical Andes forests.

Vladimir Camel is a Forestry Engineer and is currently pursuing doctoral studies in Biological Science and Engineering at the Universidad Nacional Agraria La Molina, Lima, Perú. His research interests include the molecular physiology of plants and conservation of high Andean forest ecosystems.

Edith Orellana Mendoza is a Forestry Engineer and professor in the Area of Biodiversity and Forest Management at the Faculty of Forestry and Environmental Sciences, Universidad Nacional del Centro del Peru. Her areas of interest are environmental pollution, the risks of toxics in the environment, and biodiversity.

Marcela V. Pyles is a Forestry Engineer who is currently pursuing doctoral studies in Applied Ecology at the Biology Institute, Sector of Ecology and Conservation, Federal University of Lavras (UFLA), Minas Gerais, Brazil.



Rudolf von May is an Assistant Professor in the Biology Program at California State University Channel Islands, Camarillo, California, USA. His fields of interest include evolutionary ecology and biodiversity conservation.

Appendix 1. Variables included in each of the top 14 models for size of *Rhinella yanachaga* (mm). Abbreviations: Sea = Season; Mic = Microhabitat; Ele = Elevation; Sex = Sex; df = degrees of freedom; ΔAICc = difference between the AICc of a given model and that of the best model; Wt = Akaike weights; × = interaction term. The plus signs indicate that the variable/interaction with categorical variable is included in the model.

Intercept	Sea	Mic	Ele	Sex	Sea × Mic	Sea × Ele	Sea × Sex	Mic × Ele	Mic × Sex	Ele × Sex	df	logLik	AICc	delta	weight
3.534	+	+		+							8	-635.325	1287.317	0.000	0.140
3.553		+		+							7	-636.883	1288.281	0.964	0.087
3.597	+	+		+	+						10	-633.741	1288.510	1.193	0.077
3.533	+	+	0.009	+							9	-635.029	1288.896	1.579	0.064
3.541	+	+		+			+				9	-635.263	1289.363	2.046	0.050
3.553		+	0.008	+							8	-636.631	1289.929	2.612	0.038
3.528	+	+	0.023	+		+					10	-634.465	1289.957	2.640	0.038
3.593	+	+	0.005	+	+						11	-633.635	1290.509	3.192	0.028
3.601	+	+		+	+		+				11	-633.726	1290.691	3.374	0.026
3.540	+	+	0.009	+			+				10	-634.966	1290.960	3.643	0.023
3.533	+	+	0.006	+						+	10	-635.016	1291.060	3.743	0.022
3.563	+	+		+					+		10	-635.025	1291.078	3.762	0.021
3.533	+	+	0.005	+				+			11	-634.009	1291.257	3.940	0.020
3.639	+	+	-0.064	+	+			+			13	-631.928	1291.581	4.264	0.017

Appendix 2. Comparisons of the number of individuals of *Rhinella yanachaga* in each season (wet and dry) and microhabitat (bromeliad, fern, and leaf). Asterisks represent significantly different parameters with the Tukey test after GLMM: "*" $p \leq 0.05$; "**" $p \leq 0.01$; and "***" $p \leq 0.001$.

Number of individuals			Estimate	Std. error	z value	Pr (> |z|)	
Season	Male	Wet	1.266	0.241	5.243	< 0.001	***
		Dry	-0.199	0.317	-0.628	0.53	
	Female	Wet	-1.266	0.241	-5.243	< 0.001	***
		Dry	0.199	0.317	0.628	0.53	
Microhabitat	Male	Bromeliad	0.876	0.532	1.645	0.1	
		Fern	0.338	0.585	0.577	0.564	
		Leaf	0.377	0.58	0.65	0.516	
	Female	Bromeliad	0.876	0.532	1.645	0.1	
		Fern	0.338	0.585	0.577	0.564	
		Leaf	0.377	0.58	0.65	0.516	